

a concentration-dependent manner. The inhibitory effect was fully countered by 1 mM orthovanadate (an inhibitor of protein tyrosine phosphatases). The inhibitory effect of hEAG1 current by 10 μ M AG556 (WT: $63.6 \pm 6.0\%$, $n=6$) was highly attenuated in the mutant hEAG1-Y90A ($33.7 \pm 3.7\%$, $n=7$, $P<0.001$ vs WT), Y344A ($33.1 \pm 6.0\%$, $n=5$, $P<0.005$ vs WT) and Y485A ($21.5 \pm 3.8\%$, $n=5$, $P<0.001$ vs WT), but not Y376A ($61.7 \pm 5.6\%$, $n=6$). These results demonstrate for the first that EGFR kinase modulates hEAG1 channel activity via phosphorylating tyrosine residues Tyr⁹⁰, Try³⁴⁴ and Try⁴⁸⁵ and likely regulates neuronal activity and tumor growth.

889-Pos Board B768

Modeling Of The Adrenergic Response Of The Human I_{Ks} Current (hKCNQ1/hKCNE1) Stably Expressed In HEK-293 Cells

John P. Imredy¹, Jacob R. Penniman¹, Spencer J. Dech¹, Winston D. Irving², Joseph J. Salata¹.

¹Merck & Co., Safety Assessment, West Point, PA, USA, ²Beth Israel Medical Center, New York, NY, USA.

Adrenergic enhancement of the slowly activating delayed rectifier current (I_{Ks}) in cardiac myocytes constitutes a critical "repolarization reserve". Stable co-expression of human (h)KCNQ1 and hKCNE1 in HEK-293 cells reconstitutes a native-like I_{Ks} current (HEK- I_{Ks}), allowing β -adrenergic modulation of the current by stimulation of endogenous signalling pathways in the host cell line. HEK- I_{Ks} currents were enhanced two- to fourfold by bath application of isoproterenol ($EC_{50} = 13$ nM), forskolin (10 μ M), or 8-(4-chlorophenylthio) adenosine 3',5'-cyclic monophosphate (50 μ M), indicating an intact cAMP dependent ion channel-regulating pathway analogous to that observed in native cardiac myocytes. We made use of the robust modulation of the I_{Ks} current to model in detail the effects of adrenergic modulation on I_{Ks} gating kinetics. Activation kinetics of HEK- I_{Ks} were accurately fit with a novel modified 2nd order Hodgkin-Huxley (H-H) gating model incorporating a fast and a slow gate, each independent of each other in scale and adrenergic response, or a "heterodimer" model. Macroscopically, β -adrenergic enhancement shifted HEK- I_{Ks} current activation to more negative potentials and accelerated activation kinetics, while leaving deactivation kinetics relatively unaffected. Modeling of the current in response to 10 μ M forskolin indicated that the observed changes in gating could be largely explained by modulation of the opening rate of the fast gate of the H-H model. Rate-dependent accumulation of I_{Ks} at high pulsing rates had two phases, an initial staircaselike effect, followed by a slower, incremental accumulation phase. These phases are readily interpreted in the context of a heterodimeric H-H model with two independent gates with differing closing rates. These results indicate the HEK-293 line serves as an attractive host for studies of the effects of pharmacological and genetic manipulations upon the adrenergic modulation of I_{Ks} .

890-Pos Board B769

Loss Of Transient Outward Potassium Current (Ito) Gradient Across The Ventricular Wall With Exposure To Elevated Levels Of Glucose

Keith W. Dilly¹, Fernando Santana².

¹University of Leeds, Leeds, United Kingdom, ²University of Washington, Washington, WA, USA.

In heart, pore-forming Kv4 channel subunits underlie the K^+ transient outward current (I_{to}). Expression of Kv4 is greater in left ventricular epicardial (EPI) than in endocardial (ENDO) cells, resulting in larger I_{to} in EPI than in ENDO cells. In adult myocytes, the transcription factor NFATc3 suppresses Kv4 expression. NFATc3 activity is higher in ENDO than in EPI cells and this has been proposed to contribute to heterogeneous Kv4 expression across the left ventricular free wall. It has been shown that elevated glucose activates NFATc3 in vascular smooth muscle. Here, we tested the hypothesis that elevated glucose reduces expression of I_{to} and dissipates the gradient of I_{to} density across the left ventricular free wall of mouse myocardium.

Adult murine ventricular myocytes exposed to external medium containing elevated levels of D-glucose (25 mM) for 24 hrs *in vitro* showed significant reductions in I_{to} compared with control (10 mM). Circulating blood glucose was measured in a murine model of diabetes (*db/db*). Significantly elevated levels of circulating blood glucose were found in *db/db* mice compared with control *db/db* mice. Myocytes from *db/db* mice showed a loss of transmural gradient in I_{to} density, with levels of EPI I_{to} reduced to those of ENDO I_{to} . However, a heterogeneous gradient in I_{to} was maintained in control *db/db* mice. Unlike myocytes from wild type, and *db/db* mice, myocytes from NFATc3-null mice did not undergo changes in I_{to} density during exposure to elevated glucose.

Collectively, these data suggest NFATc3 signalling contributes to the loss of heterogeneous Kv4 expression, and hence I_{to} density, in the mouse left ventricle during exposure to elevated levels of glucose. Mechanisms underlying these effects of elevated glucose on the transmural gradient of I_{to} will be discussed.

891-Pos Board B770

Effects Of Estrogen On The I_{Kr} Channel And Cardiac Repolarization

Junko Kurokawa¹, Masaji Tamagawa², Nobuhiro Harada³, Shin-ichiro Honda³, Haruaki Nakaya², Tetsushi Furukawa¹.

¹Tokyo Medical and Dental University, Medical Research Institute, Tokyo, Japan, ²Chiba University Graduate School of Medicine, Chiba, Japan,

³Fujita Health University, School of Medicine, Aichi, Japan.

Females gender itself is a risk factor for drug-induced *torsades de pointes* (TdP) arrhythmia which is associated with QT prolongation caused by blockade of human ether-a-go-go related gene (hERG) currents. Some clinical evidence suggests that estrogen is a determinant of the gender-differences in drug-induced QT prolongation and baseline QT_C intervals. Although the chronic effects of estrogen have been studied, it remains unclear whether the gender differences are due entirely to transcriptional regulations through estrogen receptors. We here found that the most bioactive estrogen, 17 β -estradiol (E2), acutely delayed cardiac repolarization within the physiological serum level (0.1-1 nM). E2 slightly but significantly suppressed hERG currents ($K_d = 0.6$ nM) by modifying channel gating kinetics. Mutagenesis study showed the interaction of E2 with F656, a common drug-binding site at the inner pore-cavity of hERG. E2 enhanced both hERG suppression and QT_C prolongation by its blocker, E4031. The lack of effects of testosterone on hERG currents and E4031-sensitivity implicates the critical role of aromatic centroid present in E2 but not in testosterone, which is supported by data from aromatase-null mice that cannot produce estrogen. The aromatase-null mice showed lower sensitivity to E4031-induced QT prolongation compared with those of wild type mice, and *i.v.* application of exogenous E2 (0.1 μ g/kg) subsequent to E4031 administration rapidly prolonged QT intervals, indicating that aromatized estrogen emphasize the effect of E4031 on cardiac repolarization *in vivo*. Our data indicate that E2 acutely affects the hERG channel gating and the E4031-induced QT_C prolongation, and may provide a novel mechanism for the higher susceptibility to drug-induced arrhythmia in women.

892-Pos Board B771

Four-and-a-half LIM Protein 2 And Erk1/2 Are Involved In The Regulation Of The I_{Ks} Current In The Heart

Nathalie Hélix Nielsen¹, Guiscard Seeböhm², Kirstine Calloe¹,

Nathalie Strutz-Seeböhm², Søren-Peter Olesen¹, Nicole Schmitt¹.

¹U Copenhagen, Faculty of Health Sciences, The Panum Institute, Copenhagen N, Denmark, ²Ruhr University Bochum, Department of Biochemistry I, Bochum, Germany.

Aims: The slow delayed rectifier potassium current in the heart, I_{Ks} , is important for terminating the plateau phase of the action potentials and for the repolarization of the atrial and ventricular cardiomyocytes. We set out to identify new players in cardiac repolarization that serve as regulators in the cellular network. Identification of interaction partners may allow us to understand spatial and temporal variations in ion channel function.

Methods and results: We performed a yeast two-hybrid screen of a heart cDNA library using Kv7.1 N-terminus as a bait and identified the four-and-a-half LIM domain protein 2 (FHL2) as an interacting partner. We investigated the functional consequences of this interaction by expressing Kv7.1 and FHL2 in heterologous expression systems. We performed two-electrode voltage-clamp recordings on *Xenopus laevis* oocytes and patch-clamp experiments in mammalian cells (CHO-K1). While FHL2 did not affect the expression levels of wild-type (WT) Kv7.1 or Kv7.1/KCNE1 currents (I_{Ks} channel), it recovered two LQT5 mutants I_{Ks} channel complexes (KCNE1-D76N and KCNE1-S74L) that, typically, show markedly reduced currents in heterologous expression systems. We additionally showed that mutation in the ERK1/2 (MAPK3) phosphorylation site in Kv7.1 N-terminus removes the rescuing effect of FHL2 on the I_{Ks} -D76N mutant channel.

Conclusion: With the present study, we identified two additional partners of the cardiac I_{Ks} complex that interact with Kv7.1, namely FHL2 and ERK1/2. In addition to the previously identified partners (beta-tubulin, calmodulin and Yotiao), our results show that understanding Kv7.1 intracellular regulation is important in order to comprehend the physiological effect of mutations inducing the LQT syndrome.

893-Pos Board B772

L-arginine Decreases L-type Ca²⁺ Current Through Receptor Activation Of NO-cGMP Cascade. Enigma Of "Arginine Paradox"

Miroslav N. Nenov, Kirill S. Grushin, Oleg Yu Pimenov, Vladimir V. Dynnik, Yuri M. Kokoz.

ITEB RAS, Pushchino, Russian Federation.

One of the most important problems, related to synthesis of NO, is the problem of "arginine paradox". In preliminary studies we demonstrated, that arginine paradox is realized not only in endothelial cells, but also in isolated cardiomyocytes. The aim of this study was to investigate receptor hypothesis of "arginine paradox" formation in isolated rat cardiomyocytes. Thus we studied the